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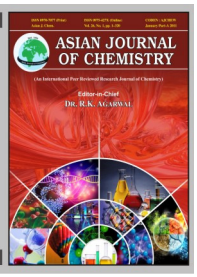

O- and *N*-Substituted Derivatives of Planetol as Valuable Bioactive Compounds

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The compounds bearing sulfamoyl and acetamoyl groups have been found to show various biological activities. In the present research work, a series of *O*- and *N*-substituted derivatives were synthesized, starting with planetol (**1**). First *N*-methyl-4-hydroxyanilinium sulfate (**1**; planetol or metol) was treated with different aryl sulfonyl chlorides (**2a-i**) using aq. sodium carbonate solution as reaction medium to yield *N*-substituted derivatives **3a-i**. The electrophile, *N*-(2,3-dihydro-1,4-benzodioxin-6-yl)-2-bromoacetamide (**5**) was prepared by the reaction of 2,3-dihydro-1,4-benzodioxin-6-amine (**4**) and 2-bromoacetylbromide in a weak basic aqueous medium. The target *O*-substituted molecules **6a-i**, were synthesized by gearing up the electrophile **5**, with the molecules **3a-i**, in a polar aprotic solvent using LiH as an activator. The proposed structures of all the synthesized molecules were corroborated by IR, ^{1}H NMR and EIMS spectral data. The *in vitro* enzyme inhibition and antibacterial studies rendered the synthesized molecules as better cholinesterase inhibitors and moderately better antibacterial agents. To explore the binding modes of the synthesized compounds, all of them were computationally docked against the active sites of acetyl cholinesterase (AChE), butyryl cholinesterase (BChE) and lipoxygenase (LOX). The compounds showed significant interactions and good correlation with the experimental data.

Keywords: 2,3-Dihydro-1,4-benzodioxin-6-amine, Antibacterial activity, Enzyme inhibition, Planetol, Computational methodology.

INTRODUCTION

Acetyl cholinesterase (AChE, EC 3.1.1.7) and butyryl cholinesterase (BChE, EC 3.1.1.8) are the parts of serine hydrolase enzymes. These are capable to terminate acetylcholine at cholinergic synapses. Because of the components of cholinergic brain synapses and neuromuscular junctions, these catalyze the hydrolysis of the neurotransmitter acetylcholine and so terminate the nerve impulse in cholinergic synapses[1]. These cholinesterase inhibitors enhance acetylcholine for neuronal and neuromuscular transmission reversibly or irreversibly[2]. In lipoxygenase type-1 (LOX, EC 1.13.11.12), the Fe^{2+} is oxidized to the catalytically active Fe^{3+} by the reaction product 15-hydroperoxy-eicosatetraenoic acid (15-HPETE) and leukotrienes from arachidonic acid as a substrate and 13-hydroperoxy-octadecadienoic acid (13-HPODE) from linoleic acid as a substrate[3]. Leukotrienes are important biologically active mediators in variety of inflammatory events. It has been found that these lipoxygenase products play a key role in a variety of disorders such as bronchial asthma inflammation[4].

The synthesis of the compounds bearing poly-functional groups has been increasing interest these days. In the demoed work, molecules bearing sulfamoyl and acetamoyl functionalities attached to 1,4-benzodioxane ring have been prepared in good yields. The 1,4-benzodioxane moiety has valuable importance in biological active compounds like silybin[5], americanin A[6] and haedoxan A[7] has antihepato-toxic and insecticidal activity. The sulfonamides have gained interest of researchers because of antimicrobial, antiinflammatory, antithyroid, anticancer, antitumor, antiviral, *etc*. activities and as inhibitors of carbonic anhydrase, cystein protease, cyclohydrogenase, HIV protease, *etc*.[8-11]; and acetamides-possess anthelmintic, anticonvulsant, antioxidant, antiinflammatory, antiarthritic, anticancer, antibacterial and antifungal activities[12-15].

The synthesis of various sulfonamides and acetamides, to inaugurate new less toxic and more efficient molecules, prompted us to take into account the synthesis of such molecules bearing both the moieties. The present work is the protraction of our successful efforts for the synthesis of potent bioactive sulfonamides, acetamides and heterocyclic compounds[16-19]. The series of synthesized molecules were found to

be potentially more active against cholinesterase enzymes, moderate against Gram-bacterial strains and less against lipoxygenase enzyme.

The docking method involves the prediction of ligand conformation and orientation with in a targeted binding site. Generally, there are two objectives of docking studies *viz.*, precise structural modeling and accurate prediction of activity[20]. Molecular docking can be used to explain the mechanism of molecular recognition between the ligands and its receptor. Some theoretic docking methods have been used to study the interactions of molecular recognition. Among them one is MOE-Dock[21] method which allows the ligands to be flexible during docking so that the ligands can adjust their different conformations in the binding pocket of the receptor. We have applied this method to find the best binding mode between the synthesized compounds and cholinesterase (acetyl cholinesterase, butyryl cholinesterase) and lipoxygenase.

EXPERIMENTAL

Melting points of the synthesized compounds were recorded on open capillary tube Griffin and George melting point apparatus and were uncorrected. Purity was checked out by thin layer chromatography (layer chromatography) on pre-coated silica gel G-25-UV254 plates with different polarity solvent systems using ethyl acetate and *n*-hexane giving single spot. Identification of spots was carried out at 254 nm UV lamp and by ceric sulphate reagent on heating. The IR spectra were recorded in KBr pellet method on a Jasco-320-A spectrophotometer with wave number in cm^{-1}. Nuclear magnetic resonance spectra were recorded in CD_3OD on a Bruker spectrometers operating at 300 MHz. Chemical shifts are given in ppm taking TMS as internal reference standard and coupling constant (*J*) in Hz. The abbreviations used in ^1H NMR were s = singlet, d = doublet and dd = doublet of doublet. Mass spectra (EIMS) were recorded on a JMS-HX-110 spectrometer, with a data system. All the spectra were taken at 25 °C (r. temp.). All the chemicals taken into account were purchased from Alfa Aesar, Sigma Aldrick and Merck through local suppliers. The solvents used in the discussed project were of analytical grade.

General procedure for the synthesis of *N*-substituted derivatives of planetol (3a-i): *N*-methyl-4-hydroxyanilinium sulfate (1;10 mmol) was dissolved in 25 mL distilled water in a 100 mL round bottom flask and basified to pH of 8-10 by 10 % aqueous Na_2CO_3 solution. The aryl sulfonyl chlorides (**2a-i**; 10 mmol) were added to the reaction medium gradually in 5-10 min. The reaction mixture was stirred for 4-5 h along with monitoring by layer chromatography till the single spot. At the end of reaction, dil. HCl (2 mL) was added slowly along with vigorous hand shaking to adjust the pH to 3-5. The precipitates were generated after a stay of 5-10 min which were filtered, washed with distilled water and dried to afford the title solid compounds, **3a-i** in good yields. Recrystallization was progressed in methanol.

***N*-(4-Hydroxyphenyl)-*N*-methylbenzenesulfonamide (3a):** Light brown amorphous solid; Yield: 92 %; m.p. 106 °C; m.f.: $C_{13}H_{13}NO_3S$; m.w.: 263 g mol^{-1}; HR-MS: $[M]^+$ 263.3132 (Calcd. for $C_{13}H_{13}NO_3S$; 263.3361); IR (KBr, ν_{max}, cm^{-1}): 3365 (O-H stretching), 3061 (C-H stretching of aromatic ring), 2923 (-CH_2- stretching), 1623 (C=C stretching of aromatic ring), 1376 (-SO_2 stretching), 1154 (C-O-C stretching of ether); ^1H NMR (CD_3OD, 300 MHz): δ (ppm) 7.64 (brd, *J* = 9.0 Hz, 2H, H-2' & H-6'), 7.62-7.51 (m, 3H, H-3' to H-5'), 6.84 (d, *J* = 8.7 Hz, 2H, H-2 & H-6), 6.67 (d, *J* = 8.7 Hz, 2H, H-3 & H-5), 3.11 (s, 3H, CH_3-1''), EIMS: *m/z* 263 $[M]^+$, 199 $[M\text{-}SO_2]^+$, 141 $[C_6H_5SO_2]^+$, 122 $[C_7H_8NO]^+$, 93 $[C_6H_5O]^+$, 77 $[C_6H_5]^+$, 51 $[C_4H_3]^+$.

***N*-(4-Hydroxyphenyl)-*N*,4-dimethylbenzenesulfonamide (3b):** Light grey amorphous solid; Yield: 93 %; m.p. 125 °C; m.f.: $C_{14}H_{15}NO_3S$; m.w.: 277 g mol^{-1}; HR-MS: $[M]^+$ 277.3403 (Calcd. for $C_{14}H_{15}NO_3S$; 277.3610); IR (KBr, ν_{max}, cm^{-1}): 3356 (O-H stretching), 3059 (C-H stretching of aromatic ring), 2937 (-CH_2- stretching), 1604 (C=C stretching of aromatic ring), 1378 (-SO_2 stretching), 1145 (C-O-C stretching of ether); ^1H NMR (CD_3OD, 300 MHz): δ (ppm) 7.42 (d, *J* = 8.4 Hz, 2H, H-2' & H-6'), 7.34 (d, *J* = 8.1 Hz, H-3' & H-5'), 6.84 (d, *J* = 8.7 Hz, 2H, H-2 & H-6), 6.67 (d, *J* = 8.7 Hz, 2H, H-3 & H-5), 3.01 (s, 3H, CH_3-1''), 2.41 (s, 3H, CH_3-4'); EIMS: *m/z* 277 $[M]^+$, 213 $[M\text{-}SO_2]^+$, 155 $[CH_3C_6H_5SO_2]^+$, 122 $[C_7H_8NO]^+$, 93 $[C_6H_5O]^+$, 91 $[CH_3C_6H_5]^+$, 50 $[C_4H_2]^+$.

4-(*tert*-Butyl)-*N*-(4-hydroxyphenyl)-*N*-methylbenzenesulfonamide (3c): Blackish brown amorphous solid; Yield: 90 %; m.p. 76 °C; m.f.: $C_{17}H_{21}NO_3S$; m.w.: 319 g mol^{-1}; HR-MS: $[M]^+$ 319.4207 (Calcd. for $C_{17}H_{21}NO_3S$; 319.4371); IR (KBr, ν_{max}, cm^{-1}): 3355 (O-H stretching), 3066 (C-H stretching of aromatic ring), 2913 (-CH_2- stretching), 1615 (C=C stretching of aromatic ring), 1390 (-SO_2 stretching), 1167 (C-O-C stretching of ether); ^1H NMR (CD_3OD, 300 MHz): δ (ppm) 7.58 (d, *J* = 8.7 Hz, 2H, H-2' & H-6'), 7.47 (d, *J* = 8.4 Hz, H-3' & H-5'), 6.86 (d, *J* = 9 Hz,2H, H-2 & H-6), 6.68 (d, *J* = 8.7 Hz, 2H, H-3 & H-5), 3.10 (s, 3H, CH_3-1''), 1.36 (s, 9H, $(CH_3)_3$C-4'); EIMS: *m/z* 319 $[M]^+$, 255 $[M\text{-}SO_2]^+$, 197 $[(CH_3)_3CC_6H_5SO_2]^+$, 133 $[(CH_3)_3CC_6H_5]^+$, 122 $[C_7H_8NO]^+$, 93 $[C_6H_5O]^+$, 50 $[C_4H_2]^+$.

***N*-(4-Hydroxyphenyl)-*N*-2,4,6-tetramethylbenzenesulfonamide (3d):** Black amorphous solid; Yield: 84 %; m.p. 60 °C; m.f.: $C_{16}H_{19}NO_3S$; m.w.: 305 g mol^{-1}; HR-MS: $[M]^+$ 305.3934 (Calcd. for $C_{16}H_{19}NO_3S$; 305.4106); IR (KBr, ν_{max}, cm^{-1}): 3364 (O-H stretching), 3069 (C-H stretching of aromatic ring), 2924 (-CH_2- stretching), 1604 (C=C stretching of aromatic ring), 1395 (-SO_2 stretching), 1159 (C-O-C stretching of ether); ^1H NMR (CD_3OD, 300 MHz): δ (ppm) 6.95 (s, 2H, H-3' & H-5'), 6.92 (d, *J* = 8.7 Hz, 2H, H-2 & H-6), 6.65 (d, *J* = 9 Hz, 2H, H-3 & H-5), 3.19 (s, 3H, CH_3-1''), 2.38 (s, 6H, CH_3-2' & 6'), 2.27 (s, 3H, CH_3-4'); EIMS: *m/z* 305 $[M]^+$, 241 $[M\text{-}SO_2]^+$, 183 $[(CH_3)_3C_6H_5SO_2]^+$, 122 $[C_7H_8NO]^+$, 119 $[(CH_3)_3C_6H_5]^+$, 93 $[C_6H_5O]^+$.

4-(Acetylamino)-*N*-(4-hydroxyphenyl)-*N*-methylbenzenesulfonamide (3e): Grayish brown amorphous solid; Yield: 91 %; m.p. 152 °C; m.f.: $C_{15}H_{16}N_2O_4S$; m.w.: 320 g mol^{-1}; HR-MS: $[M]^+$ 320.3654 (Calcd. for $C_{15}H_{16}N_2O_4S$; 320.3849); IR (KBr, ν_{max}, cm^{-1}): 3369 (O-H stretching), 3073 (C-H stretching of aromatic ring), 2927 (-CH_2- stretching), 1613 (C=C stretching of aromatic ring), 1379 (-SO_2 stretching), 1153 (C-O-C stretching of ether); ^1H NMR (CD_3OD, 300 MHz): δ (ppm) 7.72 (d, *J* = 8.7 Hz, 2H, H-3' & H-5'), 7.47 (d,

J = 8.7 Hz, 2H, H-2' & H-6'), 6.86 (d, *J* = 8.7 Hz, 2H, H-2 & H-6), 6.68 (d, *J* = 9 Hz, 2H, H-3 & H-5), 3.10 (s, 3H, $\underline{CH_3}CONH$-4'), 2.14 (s, 3H, CH_3-1"); EIMS: *m/z* 320 $[M]^+$, 256 $[M\text{-}SO_2]^+$, 198 $[CH_3CONHC_6H_5SO_2]^+$, 134 $[CH_3CONHC_6H_5]^+$, 122 $[C_7H_8NO]^+$, 93 $[C_6H_5O]^+$, 50 $[C_4H_2]^+$.

4-Bromo-*N*-(4-hydroxyphenyl)-*N*-methylbenzen-esulfonamide (3f): Dark brown amorphous solid; Yield: 90 %; m.p. 78 °C; m.f.: $C_{13}H_{12}NO_3SBr$; M.w.: 341 g mol^{-1}; HR-MS: $[M]^+$ 341.2091 (Calcd. for $C_{13}H_{12}BrNO_3S$; 341.2235); IR (KBr, ν_{max}, cm-1): 3354 (O-H stretching), 3051 (C-H stretching of aromatic ring), 2926 (-CH_2- stretching), 1608 (C=C stretching of aromatic ring), 1372 (-SO_2 stretching), 1147 (C-O-C stretching of ether); ^{1}H NMR (CD_3OD, 300 MHz): δ (ppm) 7.71 (d, *J* = 8.7 Hz, 2H, H-2' & H-6'), 7.44 (d, *J* = 8.4 Hz, 2H, H-3' & H-5'), 6.87 (d, *J* = 9 Hz, 2H, H-2 & H-6), 6.69 (d, *J* = 9 Hz, 2H, H-3 & H-5), 3.12 (s, 3H, CH_3-1"); EIMS: *m/z* 343 $[M + 2]^+$, 341 $[M]^+$, 277 $[M\text{-}SO_2]^+$, 219 $[BrC_6H_5SO_2]^+$, 155 $[BrC_6H_5]^+$, 122 $[C_7H_8NO]^+$, 93 $[C_6H_5O]^+$, 50 $[C_4H_2]^+$.

4-Chloro-*N*-(4-hydroxyphenyl)-*N*-methylbenzenesulfonamide (3g): Blackish grey amorphous solid; Yield: 94 %; m.p. 100 °C; m.f.: $C_{13}H_{12}NO_3SCl$; m.w.: 297 g mol^{-1}; HR-MS: $[M]^+$ 297.3586 (Calcd. for $C_{13}H_{12}NO_3SCl$; 297.3739); IR (KBr, ν_{max}, cm^{-1}): 3351 (O-H stretching), 3070 (C-H stretching of aromatic ring), 2931 (-CH_2- stretching), 1607 (C=C stretching of aromatic ring), 1379 (-SO_2 stretching), 1178 (C-O-C stretching of ether); ^{1}H NMR (CD_3OD, 300 MHz): δ (ppm) 7.53 (d, *J* = 8.1 Hz, 2H, H-2' & H-6'), 7.45 (d, *J* = 8.1 Hz, 2H, H-3' & H-5'), 6.87 (d, *J* = 8.4 Hz, 2H, H-2 & H-6), 6.69 (d, *J* = 8.7 Hz, 2H, H-3 & H-5), 3.12 (s, 3H, CH_3-1"); EIMS: *m/z* 299 $[M + 2]^+$, 297 $[M]^+$, 233 $[M\text{-}SO_2]^+$, 175 $[ClC_6H_5SO_2]^+$, 122 $[C_7H_8NO]^+$, 111 $[ClC_6H_5]^+$, 93 $[C_6H_5O]^+$, 50 $[C_4H_2]^+$.

2,5-Dichloro-*N*-(4-hydroxyphenyl)-*N*-methylbenzenesulfonamide (3h): Blackish grey amorphous solid; yield: 87 %; m.p. 76 °C; m.f.: $C_{13}H_{11}NO_3SCl_2$; mole-cular weight: 331 g mol^{-1}; HR-MS: $[M]^+$ 331.2035 (Calcd. for $C_{13}H_{11}Cl_2NO_3S$; 331.2148); IR (KBr, ν_{max}, cm^{-1}): 3375 (O-H stretching), 3072 (C-H stretching of aromatic ring), 2937 (-CH_2- stretching), 1617 (C=C stretching of aromatic ring), 1379 (-SO_2 stretching), 1170 (C-O-C stretching of ether); ^{1}H NMR (CD_3OD, 300 MHz): δ (ppm) 7.81 (s, 1H, H-6'), 7.71 (d, *J* = 8.4, 1H, H-3'), 7.58 (d, *J* = 8.4, 1H, H-4'), 6.87 (d, *J* = 8.7 Hz, 2H, H-2 & H-6), 6.69 (d, *J* = 9 Hz, 2H, H-3 & H-5), 3.12 (s, 3H, CH_3-1"); EIMS: *m/z* 335 $[M + 4]^+$, 333 $[M + 2]^+$, 331 $[M]^+$, 267 $[M\text{-}SO_2]^+$, 209 $[Cl_2C_6H_5SO_2]^+$, 145 $[Cl_2C_6H_5]^+$, 122 $[C_7H_8NO]^+$, 93 $[C_6H_5O]^+$.

N-(4-Hydroxyphenyl)-*N*-methyl-2-naphthalenesulfonamide (3i): Dark brown amorphous solid; Yield: 95 %; m.p. 72 °C; m.f.: $C_{17}H_{15}NO_3S$; m.w.: 313 gmol^{-1}; HR-MS: $[M]^+$ 313.3725 (Calcd. for $C_{17}H_{15}NO_3S$; 313.3834); IR (KBr, ν_{max}, cm^{-1}): 3347 (O-H stretching), 3058 (C-H stretching of aromatic ring), 2927 (-CH_2- stretching), 1609 (C=C stretching of aromatic ring), 1391 (-SO_2 stretching), 1164 (C-O-C stretching of ether); ^{1}H NMR (CD_3OD, 300 MHz): δ (ppm) 8.14 (s, 1H, H-1'), 7.96-7.90 (m, 2H, H-4' & H-5'), 7.87 (d, *J* = 8.1 Hz, 1H, H-3'), 7.64 (dd, *J* = 8.1, 2.1 Hz, 1H, H-8'), 7.52-7.47 (m, 2H, H-6' & H-7'), 6.85 (d, *J* = 8.7 Hz, 2H, H-2 & H-6), 6.66 (d, *J* = 8.7 Hz, 2H, H-3 & H-5), 3.16 (s, 3H, CH_3-1"); EIMS: *m/z* 313 $[M]^+$, 249 $[M\text{-}SO_2]^+$, 191 $[C_{10}H_7SO_2]^+$, 127 $[C_{10}H_7]^+$, 122 $[C_7H_8NO]^+$, 93 $[C_6H_5O]^+$.

Procedure for the synthesis of *N*-(2,3-dihydro-1,4-benzodioxin-6-yl)-2-bromoacetamide (5): 2,3-Dihydro-1,4-benzodioxin-6-amine (**4**; 110 mmol) was suspended in 100 mL distilled water in a 250 mL iodine flask and 10 % Na_2CO_3 solution was added to adjust the pH 8-10. The reaction mixture was hand shaked for 5-10 min and then 2-bromoacetyl-bromide (110 mmol) was poured drop wise along with vigorous shaking till the formation of solid precipitates. After complete addition, the iodine flask was kept on stirring for further 15-20 min. The solid precipitates were verified through thin layer chromatography giving single spot and acidified up to pH of 3-5. The precipitates were filtered, washed with distilled water and dried to yield the electrophile **5**. Purplish grey amorphous solid; Yield: 95 %; m.p. 56 °C; m.f.: $C_{10}H_{10}NO_3Br$; m.w.: 271 g mol^{-1}; HR-MS: $[M]^+$ 271.0952 (Calcd. for $C_{10}H_{10}BrNO_3$; 271.1076); IR (KBr, ν_{max}, cm^{-1}): 3460 (N-H stretching), 3067 (C-H stretching of aromatic ring), 2919 (-CH_2- stretching), 1618 (C=C stretching of aromatic ring), 1157 (C-O-C stretching of ether); ^{1}H NMR (CD_3OD, 300 MHz): δ (ppm) 10.20 (s, 1H, NH), 7.20 (d, *J* = 2.1 Hz, 1H, H-5), 6.95 (dd, *J* = 8.7, 2.1 Hz, 1H, H-7), 6.80 (d, *J* = 8.7 Hz, 1H, H-8), 4.19 (s, 4H, CH_2-2 & CH_2-3), 3.97 (s, 2H, CH_2-2'); EIMS: *m/z* 273 $[M + 2]^+$, 271 $[M]^+$, 150 $[C_8H_8NO_2]^+$, 135 $[C_8H_7O_2]^+$, 122 $[C_6H_4NO_2]^+$, 121 $[C_2H_2BrO]^+$, 107 $[C_6H_3O_2]^+$, 66 $[C_4H_4N]^+$.

General procedure for the synthesis of *O*-substituted derivatives (6a-i): *N*-(4-hydroxyphenyl)-*N*-methylarylsulfonamides (**3a-i**; 10 mmol) were homogeneously dissolved in *N*,*N*-dimethylformamide (DMF; 10 mL) in a 100 mL RB flask and activated by the addition of lithium hydride (10 mmol). The mixture was stirred for 15-25 min and then the calculated equimolar amount of electrophile, *N*-(2,3-dihydro-1,4-benzodioxin-6-yl)-2-bromoacetamide (**5**) was added to reaction mixture and further stirred for 5-6 h. The reaction progress was supervised via layer chromatography till single spot. Aqueous Na_2CO_3 solutionwas added to the flask and hand shaked slowly. The target precipitated products were collected by filtration, washed with distilled water and dried.

2-{4-[Methyl(phenylsulfonyl)amino]phenoxy}-*N*-(2,3-dihydro-1,4-benzodioxin-6-yl)acetamide (6a): Light brown amorphous solid; Yield: 81 %; m.p. 157 °C; m.f.: $C_{23}H_{22}N_2O_6S$; m.w.: 454 g mol^{-1}; HR-MS: $[M]^+$ 454.4975 (Calcd. for $C_{23}H_{22}N_2O_6S$; 454.5031); IR (KBr, ν_{max}, cm^{-1}): 3462 (N-H stretching), 3060 (C-H stretching of aromatic ring), 2921 (-CH_2- stretching), 1621 (C=C stretching of aromatic ring), 1381 (-SO_2 stretching), 1159 (C-O-C stretching of ether); ^{1}H NMR (CD_3OD, 300 MHz): δ (ppm) 7.64 (dd, *J* = 7.8, 1.8 Hz, 2H, H-2' & H-6'), 7.51-7.41 (m, 3H, H-3' to H-5'), 7.17 (d, *J* = 2.4, 1H, H-5'"), 7.02 (dd, *J* = 8.4, 1.2 Hz, 1H, H-7'"), 6.99 (d, *J* = 7.5 Hz, 1H, H-8'"), 6.97 (d, *J* = 8.1 Hz, 2H, H-2 & H-6), 6.78 (d, *J* = 8.7 Hz, 2H, H-3 & H-5), 4.21 (s, 4H, CH_2-2'" & CH_2-3'"), 3.92 (s, 2H, CH_2-2""), 3.14(s, 3H, CH_3-1"); EIMS: *m/z* 454 $[M]^+$, 390 $[M\text{-}SO_2]^+$, 150 $[C_8H_8NO_2]^+$, 141 $[C_6H_5SO_2]^+$, 135 $[C_8H_7O_2]^+$, 122 $[C_6H_4NO_2]^+$, 122 $[C_7H_8NO]^+$, 107 $[C_6H_3O_2]^+$, 93 $[C_6H_5O]^+$, 77 $[C_6H_5]^+$, 66 $[C_4H_4N]^+$, 51 $[C_4H_3]^+$.

2-(4-{Methyl[(4-methylphenyl)sulfonyl]amino} phenoxy)-*N*-(2,3-dihydro-1,4-benzodioxin-6-yl)acetamide (6b): Light grey amorphous solid; Yield: 80 %; m.p. 155 °C; m.f.:

$C_{24}H_{24}N_2O_6S$; m.w.: 468 g mol^{-1}; HR-MS: $[M]^+$ 468.5234 (Calcd. for $C_{24}H_{24}N_2O_6S$; 468.5364); IR (KBr, ν_{max}, cm^{-1}): 3464 (N-H stretching), 3073 (C-H stretching of aromatic ring), 2911 (-CH_2- stretching), 1610 (C=C stretching of aromatic ring), 1377 (-SO_2 stretching), 1149 (C-O-C stretching of ether); ^{1}H NMR (CD_3OD, 300 MHz): δ (ppm) 7.45 (d, *J* = 8.1 Hz, 2H, H-2' & H-6'), 7.38 (d, *J* = 8.4 Hz, H-3' & H-5'), 7.19 (d, *J* = 2.4, 1H, H-5'''), 7.06 (dd, *J* = 8.1, 1.5 Hz, 1H, H-7'''), 6.89 (d, *J* = 8.1 Hz, 2H, H-2 & H-6), 6.72 (d, *J* = 7.5 Hz, 1H, H-8'''), 6.69 (d, *J* = 8.7 Hz, 2H, H-3 & H-5), 4.21 (s, 4H, CH_2-2'''& CH_2-3'''), 3.91 (s, 2H, CH_2-2''''), 3.09 (s, 3H, CH_3-1''), 2.42 (s, 3H, CH_3-4'); EIMS: *m/z* 468 $[M]^+$, 404 $[M\text{-}SO_2]^+$, 155 $[CH_3C_6H_5SO_2]^+$, 150 $[C_8H_8NO_2]^+$, 135 $[C_8H_7O_2]^+$, 122 $[C_6H_4NO_2]^+$, 122 $[C_7H_8NO]^+$, 107 $[C_6H_3O_2]^+$, 93 $[C_6H_5O]^+$, 91 $[CH_3C_6H_5]^+$, 66 $[C_4H_4N]^+$, 50 $[C_4H_2]^+$.

2-{4-[{[4-(*tert*-Butyl)phenyl]sulfonyl}(methyl)amino] phenoxy}-*N*-(2,3-dihydro-1,4-benzodioxin-6-yl)acetamide (6c): Light brown amorphous solid; Yield: 79 %; m.p. 71 °C; m.f.: $C_{27}H_{30}N_2O_6S$; m.w.: 510 g mol^{-1}; HR-MS: $[M]^+$ 510.6034 (Calcd. for $C_{27}H_{30}N_2O_6S$; 510.6166); IR (KBr, ν_{max}, cm^{-1}): 3459 (N-H stretching), 3063 (C-H stretching of aromatic ring), 2921 (-CH_2- stretching), 1621 (C=C stretching of aromatic ring), 1390 (-SO_2 stretching), 1168 (C-O-C stretching of ether); ^{1}H NMR (CD_3OD, 300 MHz): δ (ppm) 7.58 (d, *J* = 8.4 Hz, 2H, H-2' & H-6'), 7.47 (d, *J* = 8.4 Hz, H-3' & H-5'), 7.17 (d, *J* = 1.8, 1H, H-5'''), 6.95 (dd, *J* = 8.1, 1.5 Hz, 1H, H-7'''), 6.86 (d, *J* = 8.7 Hz,2H, H-2 & H-6), 6.77 (d, *J* = 8.7 Hz, 1H, H-8'''), 6.68 (d, *J* = 8.7 Hz, 2H, H-3 & H-5), 4.21 (s, 4H, CH_2-2''' & CH_2-3'''), 3.91 (s, 2H, CH_2-2''''), 3.10 (s, 3H, CH_3-1''), 1.34 (s, 9H, $(CH_3)_3$C-4'); EIMS: *m/z* 510 $[M]^+$, 446 $[M\text{-}SO_2]^+$, 197 $[(CH_3)_3CC_6H_5SO_2]^+$, 150 $[C_8H_8NO_2]^+$, 135 $[C_8H_7O_2]^+$, 133 $[(CH_3)_3CC_6H_5]^+$, 122 $[C_6H_4NO_2]^+$, 122 $[C_7H_8NO]^+$, 107 $[C_6H_3O_2]^+$, 93 $[C_6H_5O]^+$, 66 $[C_4H_4N]^+$, 50 $[C_4H_2]^+$.

2-{4-[(Mesitylsulfonyl)(methyl)amino]phenoxy}-*N*-(2,3-dihydro-1,4-benzodioxin-6-yl)acetamide (6d): Black sticky solid; Yield: 81 %; m.f.: $C_{26}H_{28}N_2O_6S$; m.w.: 496 g mol^{-1}; HR-MS: $[M]^+$ 496.5764 (Calcd. for $C_{26}H_{28}N_2O_6S$; 496.5824); IR (KBr, ν_{max}, cm^{-1}): 3468 (N-H stretching), 3062 (C-H stretching of aromatic ring), 2936 (-CH_2- stretching), 1625 (C=C stretching of aromatic ring), 1395 (-SO_2 stretching), 1162 (C-O-C stretching of ether); ^{1}H NMR (CD_3OD, 300 MHz): δ (ppm) 7.11 (d, *J* = 2.4, 1H, H-5'''), 6.97 (s, 2H, H-3' & H-5'), 6.94 (dd, *J* = 8.4, 2.1 Hz, 1H, H-7'''), 6.87 (d, *J* = 8.4 Hz, 2H, H-2 & H-6), 6.76 (d, *J* = 8.1 Hz, 1H, H-8'''), 6.67 (d, *J* = 8.4 Hz, 2H, H-3 & H-5), 4.21 (s, 4H, CH_2-2''' & CH_2-3'''), 3.93 (s, 2H, CH_2-2''''), 2.99 (s, 3H, CH_3-1''), 2.38 (s, 6H, CH_3-2' & 6'), 2.27 (s, 3H, CH_3-4'); EIMS: *m/z* 496 $[M]^+$, 432 $[M\text{-}SO_2]^+$, 183 $[(CH_3)_3C_6H_5SO_2]^+$, 150 $[C_8H_8NO_2]^+$, 135 $[C_8H_7O_2]^+$, 122 $[C_6H_4NO_2]^+$, 122 $[C_7H_8NO]^+$, 119 $[(CH_3)_3C_6H_5]^+$, 107 $[C_6H_3O_2]^+$, 93 $[C_6H_5O]^+$, 66 $[C_4H_4N]^+$.

2-{4-[{[4-(Acetylamino)phenyl]sulfonyl}(methyl) amino]phenoxy}-*N*-(2,3-dihydro-1,4-benzodioxin-6-yl)acetamide (6e): Brownish grey amorphous solid; Yield: 85 %; m.p. 140 °C; m.f.: $C_{25}H_{25}N_3O_7S$; M.w.: 511 g mol^{-1}; HR-MS: $[M]^+$ 511.5488 (Calcd. for $C_{25}H_{25}N_3O_7S$; 511.5574); IR (KBr, ν_{max}, cm-1): 3467 (N-H stretching), 3034 (C-H stretching of aromatic ring), 2936 (-CH_2- stretching), 1602 (C=C stretching of aromatic ring), 1386 (-SO_2 stretching), 1162 (C-O-C stretching of ether); ^{1}H NMR (CD_3OD, 300 MHz): δ (ppm) 7.72 (d, *J* = 8.7 Hz, 2H, H-2' & H-6'), 7.46 (d, *J* = 8.5 Hz, 2H, H-3' & H-5'), 7.18 (d, *J* = 2.4, 1H, H-5'''), 6.98 (dd, *J* = 8.4, 1.8 Hz, 1H, H-7'''), 6.87 (d, *J* = 8.7 Hz,2H, H-2 & H-6), 6.77 (d, *J* = 8.1 Hz, 1H, H-8'''), 6.68 (d, *J* = 8.7 Hz, 2H, H-3 & H-5), 4.21 (s, 4H, CH_2-2''' & CH_2-3'''), 3.91 (s, 2H, CH_2-2''''), 3.11 (s, 3H, CH_3CONH-4'), 2.98 (s, 3H, CH_3-1''); EIMS: *m/z* 511 $[M]^+$, 447 $[M\text{-}SO_2]^+$, 198 $[CH_3CONHC_6H_5SO_2]^+$, 150 $[C_8H_8NO_2]^+$, 135 $[C_8H_7O_2]^+$, 134 $[CH_3CONHC_6H_5]^+$, 122 $[C_6H_4NO_2]^+$, 122 $[C_7H_8NO]^+$, 107 $[C_6H_3O_2]^+$, 93 $[C_6H_5O]^+$, 66 $[C_4H_4N]^+$, 50 $[C_4H_2]^+$.

2-{4-[[(4-Bromophenyl)sulfonyl](methyl)amino]phenoxy}-*N*-(2,3-dihydro-1,4-benzodioxin-6-yl)acetamide (6f): Light grey amorphous solid; Yield: 81 %; m.p. 83 °C; m.f.: $C_{23}H_{21}N_2O_6SBr$; M.w.: 532 g mol^{-1}; HR-MS: [M]+ 532.3937 (Calcd. for $C_{23}H_{21}N_2O_6SBr$; 532.4032); IR (KBr, cm^{-1}) ν_{max}: 3446 (N-H stretching), 3072 (C-H stretching of aromatic ring), 2908 (-CH_2- stretching), 1606 (C=C stretching of aromatic ring), 1376 (-SO_2 stretching), 1177 (C-O-C stretching of ether); 1H-NMR (CD_3OD, 300 MHz): δ (ppm) 7.76 (d, *J* = 8.7 Hz, 2H, H-2' & H-6'), 7.51 (d, *J* = 8.4 Hz, 2H, H-3' & H-5'), 7.15 (d, *J* = 2.1, 1H, H-5'''), 6.93 (dd, *J* = 8.4, 1.8 Hz, 1H, H-7'''), 6.87 (d, *J* = 8.7 Hz, 2H, H-2 & H-6), 6.77 (d, *J* = 8.1 Hz, 1H, H-8'''), 6.69 (d, *J* = 8.4 Hz, 2H, H-3 & H-5), 4.21 (s, 4H, CH_2-2''' & CH_2-3'''), 3.91 (s, 2H, CH_2-2''''), 2.98 (s, 3H, CH_3-1''); EIMS: *m/z* 534 $[M+2]^+$, 532 $[M]^+$, 468 $[M\text{-}SO_2]^+$, 219 $[BrC_6H_5SO_2]^+$, 155 $[BrC_6H_5]^+$, 150 $[C_8H_8NO_2]^+$, 135 $[C_8H_7O_2]^+$, 122 $[C_6H_4NO_2]^+$, 122 $[C_7H_8NO]^+$, 107 $[C_6H_3O_2]^+$, 93 $[C_6H_5O]^+$, 66 $[C_4H_4N]^+$, 50 $[C_4H_2]^+$.

2-{4-[[(4-Chlorophenyl)sulfonyl](methyl)amino]phenoxy}-*N*-(2,3-dihydro-1,4-benzodioxin-6-yl)acetamide (6g): Dark brown amorphous solid; Yield: 87 %; m.p. 84 °C; m.f.: $C_{23}H_{21}N_2O_6SCl$; m.w.: 488 g mol^{-1}; HR-MS: $[M]^+$ 488.5415 (Calcd. for $C_{23}H_{21}N_2O_6SCl$; 488.5516); IR (KBr, ν_{max}, cm^{-1}): 3447 (N-H stretching), 3072 (C-H stretching of aromatic ring), 2934 (-CH_2- stretching), 1606 (C=C stretching of aromatic ring), 1378 (-SO_2 stretching), 1161 (C-O-C stretching of ether); ^{1}H NMR (CD_3OD, 300 MHz): δ (ppm) 7.74 (d, *J* = 8.4 Hz, 2H, H-2' & H-6'), 7.52 (d, *J* = 8.1 Hz, 2H, H-3' & H-5'), 7.12 (d, *J* = 2.1, 1H, H-5'''), 6.95 (dd, *J* = 8.4, 2.1 Hz, 1H, H-7'''), 6.88 (d, *J* = 8.7 Hz, 2H, H-2 & H-6), 6.79 (d, *J* = 8.4 Hz, 1H, H-8'''), 6.68 (d, *J* = 8.7 Hz, 2H, H-3 & H-5), 4.21 (s, 4H, CH_2-2''' & CH_2-3'''), 3.92 (s, 2H, CH_2-2''''), 2.98 (s, 3H, CH_3-1''); EIMS: *m/z* 490 $[M + 2]^+$, 488 $[M]^+$, 424 $[M\text{-}SO_2]^+$, 175 $[ClC_6H_5SO_2]^+$, 150 $[C_8H_8NO_2]^+$, 135 $[C_8H_7O_2]^+$, 122 $[C_6H_4NO_2]^+$, 122 $[C_7H_8NO]^+$, 111 $[ClC_6H_5]^+$, 107 $[C_6H_3O_2]^+$, 93 $[C_6H_5O]^+$, 66 $[C_4H_4N]^+$, 50 $[C_4H_2]^+$.

2-{4-[[(2,5-Dichlorophenyl)sulfonyl](methyl)amino] phenoxy}-*N*-(2,3-dihydro-1,4-benzodioxin-6-yl)acetamide (6h): Black amorphous solid; Yield: 82 %; m.p. 146 °C; m.f.: $C_{23}H_{20}N_2O_6SCl_2$; m.w.: 522 g mol^{-1}; HR-MS: $[M]^+$ 522.3865 (Calcd. for $C_{23}H_{20}Cl_2N_2O_6S$; 522.3987); IR (KBr, ν_{max}, cm^{-1}): 3476 (N-H stretching), 3059 (C-H stretching of aromatic ring), 2937 (-CH_2- stretching), 1606 (C=C stretching of aromatic ring), 1393 (-SO_2 stretching), 1151 (C-O-C stretching of ether); ^{1}H NMR (CD_3OD, 300 MHz): δ (ppm) 7.81 (s, 1H, H-6'), 7.71 (d, *J* = 8.4 Hz 1H, H-3'), 7.44 (d, *J* = 8.4 Hz 1H, H-4'), 7.17 (d, *J* = 2.4, 1H, H-5'''), 6.94 (dd, *J* = 8.4, 2.4 Hz, 1H, H-7'''), 6.87 (d, *J* = 8.7 Hz, 2H, H-2 & H-6), 6.77 (d, *J* = 8.7 Hz, 1H, H-8'''), 6.69 (d, *J* = 8.7 Hz, 2H, H-3 & H-5), 4.21 (s, 4H,

CH_2-2''' & CH_2-3'''), 4.12 (s, 2H, CH_2-2''''), 3.10 (s, 3H, CH_3-1''); EIMS: *m/z* 526 $[M + 4]^+$, 524 $[M + 2]^+$, 522 $[M]^+$, 458 $[M\text{-}SO_2]^+$, 209 $[Cl_2C_6H_5\ SO_2]^+$, 150 $[C_8H_8NO_2]^+$, 145 $[Cl_2C_6H_5]^+$, 135 $[C_8H_7O_2]^+$, 122 $[C_6H_4NO_2]^+$, 122 $[C_7H_8NO]^+$, 107 $[C_6H_3O_2]^+$, 93 $[C_6H_5O]^+$, 66 $[C_4H_4N]^+$.

2-{4-[Methyl(2-naphthylsulfonyl)amino]phenoxy}-*N*-(2,3-dihydro-1,4-benzodioxin-6-yl)acetamide (6i): Light brown amorphous solid; Yield: 89 %; m.p. 90 °C; m.f.: $C_{27}H_{24}N_2O_6S$; m.w.: 504 g mol^{-1}; HR-MS: $[M]^+$ 504.5552 (Calcd. for $C_{27}H_{24}N_2O_6S$; 504.5625); IR (KBr, ν_{max}, cm^{-1}): 3475 (N-H stretching), 3070 (C-H stretching of aromatic ring), 2937 (-CH_2- stretching), 1612 (C=C stretching of aromatic ring), 1376 (-SO_2- stretching), 1182 (C-O-C stretching of ether); ^{1}H NMR (CD_3OD, 300 MHz): δ (ppm) 8.14 (s, 1H, H-1'), 7.99-7.93 (m, 2H, H-4' & H-5'), 7.67 (d, *J* = 8.1 Hz, 1H, H-3'), 7.59 (dd, *J* = 8.1, 2.1 Hz, 1H, H-8'), 7.49-7.42 (m, 2H, H-6' & H-7'), 7.17 (d, *J* = 2.4, 1H, H-5'''), 6.93 (dd, *J* = 8.7, 2.1 Hz, 1H, H-7'''), 6.85 (d, *J* = 8.7 Hz, 2H, H-2 & H-6), 6.78 (d, *J* = 8.4 Hz, 1H, H-8'''), 6.67 (d, *J* = 8.7 Hz, 2H, H-3 & H-5), 4.21 (s, 4H, CH_2-2''' & CH_2-3'''), 3.91 (s, 2H, CH_2-2''''), 3.16 (s, 3H, CH_3-1''); EIMS: *m/z* 504 $[M]^+$, 440 $[M\text{-}SO_2]^+$, 191 $[C_{10}H_7SO_2]^+$, 150 $[C_8H_8NO_2]^+$, 135 $[C_8H_7O_2]^+$, 127 $[C_{10}H_7]^+$, 122 $[C_6H_4NO_2]^+$, 122 $[C_7H_8NO]^+$, 107 $[C_6H_3O_2]^+$, 93 $[C_6H_5O]^+$, 66 $[C_4H_4N]^+$.

Cholinesterase assays: The acetyl cholinesterase and butyryl cholinesterase inhibition activities were executed according to the reported method[22] with small modifications. A volume of 100 µL comprising 60 µL Na_2HPO_4 buffer with concentration of 50 mM (pH 7.7), 10 µL test compound (0.5 mM $well^{-1}$) and 10 µL (0.005 unit $well^{-1}$ for acetyl cholinesterase and 0.5 unit $well^{-1}$ for butyryl cholinesterase) enzyme was developed. This homogeneous mixture was pre-read at 405 nm followed by pre-incubation for 10 min at 37 ºC. The reaction was started by the addition of 10 µL of 0.5 mM $well^{-1}$ substrate (acetylthiocholine iodide for acetyl cholinesterase and butyrylthiocholine chloride for BChE) and the addition of 10 µL DTNB (0.5 mM $well^{-1}$). After 15 min of incubation at 37 ºC absorbance was measured at 405 nm using 96-well plate reader Synergy HT, Biotek, USA. All experiments were carried out with their respective controls in triplicate. Eserine (0.5 mM $well^{-1}$) was used as a positive control. The percent inhibition was calculated by the following equation

$$\text{Inhibition (\%)} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

where control is the activity without inhibitor and test is the activity in the presence of test compound. IC_{50} values were calculated using EZ-Fit Enzyme kinetics software (Perrella Scientific Inc. Amherst, USA). The IC_{50} values were the average of three independent experiments.

Lipoxygenase assay: Lipoxygenase (LOX) activity was assayed according to the reported method of Baylac & Racine[23] with small modifications. Total volume of lipoxygenase assay mixture was 200 µL containing 150 µL Na_3PO_4 buffer (100 mM and pH 8), 10 µL test compound (0.5 mM $well^{-1}$) and 15 µL (600 units $well^{-1}$) enzyme. The contents were mixed, pre-read at 234 nm and pre-incubated for 10 min at 25 °C. The reaction was initiated by addition of 25 µL substrate solution. The change in absorbance was observed after 6 min at 234 nm using 96-well plate reader Synergy HT, Biotek, USA. All reactions were performed in triplicates. The positive and negative controls were included in the assay. Baicalein (0.5 mM $well^{-1}$) was used as a positive control. The percentage inhibition (%) and IC_{50} values were calculated by the same method as described cholinesterase enzymes.

Antibacterial assay: The antimicrobial activity was determined following the principle that increased absorbance of broth medium is directly related to log phase of growth and was performed in sterile 96-wells microplates under aseptic conditions[24,25]. Three Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhii*) and two Gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*) were included in the study and were maintained on stock culture agar medium. The test samples (with suitable solvents and dilutions) 20 µg/well and 180 µL fresh bacterial culture (with suitable dilution by fresh nutrient broth) was poured into wells to make a volume of 200 µL. The initial absorbance of the culture was kept 0.12-0.19 at 540 nm. The absorbance was measured at 540 nm using microplate reader, before and after incubation at 37 °C for 16-24 h with lid on the microplate. The difference was related to bacterial growth. The percent inhibition was calculated using the formula,

$$\text{Inhibition (\%)} = \frac{X - Y}{X} \times 100$$

where, X is absorbance in control with bacterial culture and Y is absorbance in test sample. Results are mean of triplicate (n = 3, ± sem). Ciprofloxacin was taken as reference standard. Minimum inhibitory concentration (MIC) was measured with suitable dilutions (5-30 µg/ well) and results were calculated using EZ-Fit Perrella Scientific Inc. Amherst USA software.

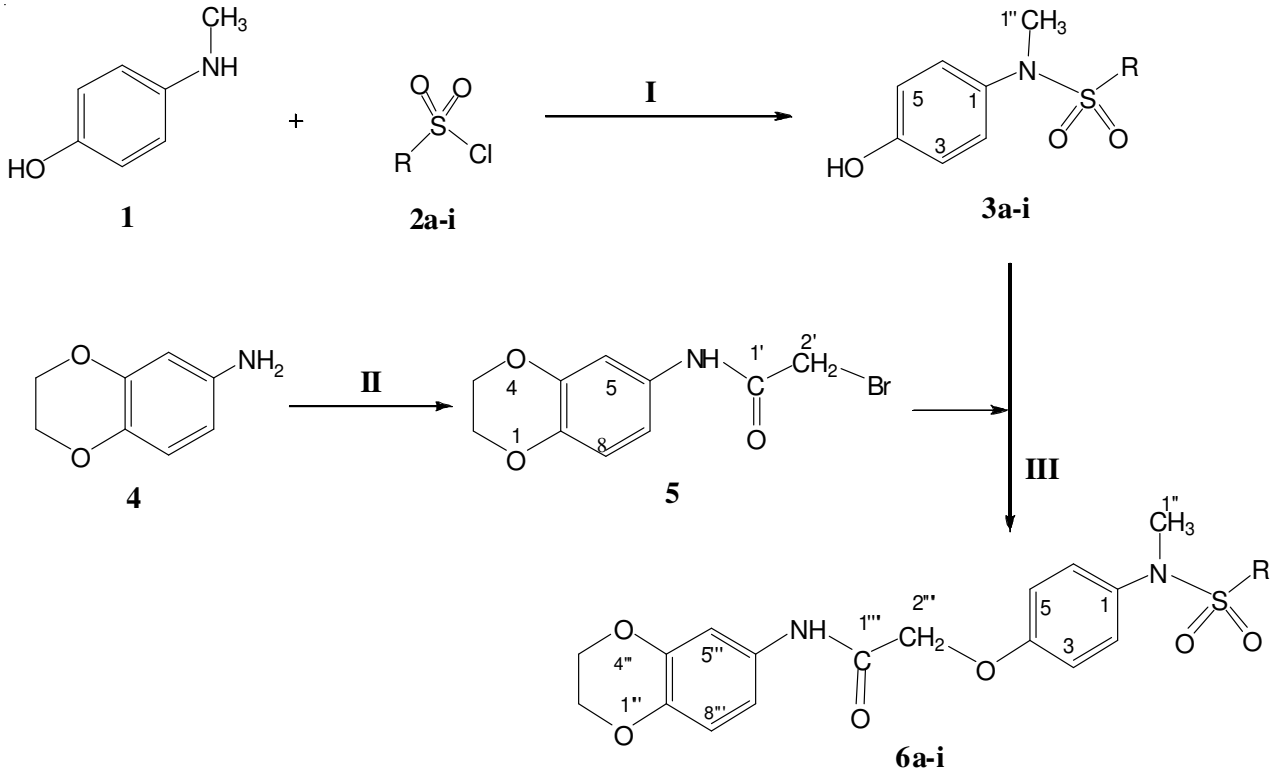
Statistical analysis: All the measurements were executed in triplicate and statistical analysis was performed by microsoft excel 2010. Results are presented as mean ± sem.

Protein preparation: The protein molecules included in our study, acetyl cholinesterase, butyryl cholinesterase and lipoxygenase were retrieved from protein data bank. Water molecules were removed and the 3D protonation of the protein molecule was performed using MOE applications. The energy of the protein molecules were minimized *via* energy minimization algorithm of MOE tool. The following parameters were used for energy minimization; gradient: 0.05, Force Field: MMFF94X+Solvation, Chiral Constraint: Current Geometry. Energy minimization was terminated when the root mean square gradient falls below the 0.05. The minimized structure was used as the template for Docking.

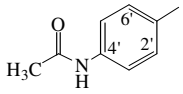
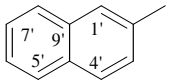
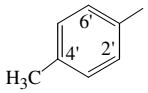
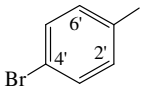
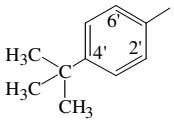
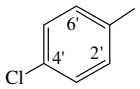
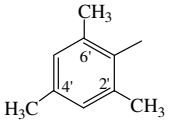
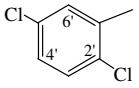
Molecular docking: The binding mode of the ligands into the binding pocket of protein molecule was predicted by MOE-Dock implemented in MOE. After the completion of docking we analyze the best poses for hydrogen bonding/π-π interactions by using MOE applications[21].

RESULTS AND DISCUSSION

The sulfamoyl and acetamoyl functionalities have been successfully geared up through a series of benignant methods by the protocol sketched in **Scheme-I**. All the procedures with reaction conditions are mentioned in experimental section. The synthesized molecules were evaluated for the anti-enzymatic and antibacterial activities and found to be valuable potent



Reagents and conditions: (**I**) Na_2CO_3/H_2O, stirring for 4-5 h, pH = 8-10 (**II**) Na_2CO_3/H_2O, 2-bromoacetyl bromide stirring for 30 min, pH = 8-10 (**III**) LiH/DMF, stirring for 5-6 h

Compd.	R	Compd.	R	Compd.	R
3a,6a		**3e,6e**		**3i,6i**	
3b,6b		**3f.6f**			
3c,6c		**3g,6g**			
3d,6d		**3h,6h**			

Scheme-I: Synthesis of *O*- and *N*-substituted derivatives of planetol

molecules as evident from their IC_{50} (Table-1) and MIC (Table-2) values respectively.

The presented research work is comprised of three major steps. First *N*-(4-hydroxyphenyl)-*N*-methylarylsulfonamides (**3a-i**) were synthesized by the reaction of *N*-methyl-4-hydroxyanilinium sulfate (**1**) and different aryl sulfonyl chlorides (**2a-i**) in a weak basic aqueous medium. The reaction was processed in basic media with pH of 8-10 to avoid the capturing of lone pair present at nitrogen by the acid produced which suppresses the nucleophilic character and also the rate of reaction. The products were separated after acidification to get rid of unreacted amine and also to enhance the yield but excess of it was obviated because of decrement in yield. The synthesized molecules **3a-i**, were further treated with *N*-(2,3-dihydro-1,4-benzodioxin-6-yl)-2-bromoacetamide (**5**) to yield the final products **6a-i**, in a weak basic aprotic polar medium. The products **6a-i**, were collected after basifying the reaction mixture in order to remove unreacted **3a-i** molecules. The electrophile **5**, was prepared by the vigorous reaction of 2,3-dihydro-1,4-benzodioxin-6-amine (**4**) with the 2-bromoacetyl bromide in a basic medium and was also collected after acidification. The structures of all the synthesized compounds

TABLE-1
PERCENTAGE INHIBITION AND IC_{50} VALUES FOR ENZYME INHIBITION ACTIVITY OF SYNTHESIZED MOLECULES

Compd	AChE		BChE		LOX	
	Inhibition (%)	IC_{50} (μM)	Inhibition (%)	IC_{50} (μM)	Inhibition (%)	IC_{50} (μM)
3a	70.75 ± 1.11	153.83 ± 0.82	51.82 ± 0.52	489.12 ± 0.59	37.92 ± 0.17	-
3b	93.49 ± 1.22	21.62 ± 0.53	88.88 ± 0.96	170.10 ± 0.62	37.71 ± 0.16	-
3c	93.41 ± 0.81	59.10 ± 0.61	88.41 ± 1.13	66.30 ± 0.89	73.29 ± 0.65	365.5 ± 0.32
3d	89.92 ± 0.91	135.22 ± 0.69	81.18 ± 0.89	187.54 ± 0.74	44.79 ± 0.45	>500
3e	79.88 ± 0.86	135.62 ± 0.32	79.53 ± 0.97	208.33 ± 0.72	66.46 ± 0.12	308.1 ± 0.75
3f	85.24 ± 0.86	62.81 ± 0.63	81.53 ± 1.19	239.64 ± 0.84	73.02 ± 0.16	194.3 ± 0.54
3g	93.53 ± 1.21	90.52 ± 0.82	90.82 ± 0.93	140.21 ± 0.47	93.71 ± 0.82	174.7 ± 0.35
3h	87.82 ± 1.16	132.86 ± 0.75	86.88 ± 1.12	221.42 ± 0.69	93.75 ± 0.42	169.3 ± 0.86
3i	94.92 ± 0.91	93.13 ± 0.62	85.18 ± 0.92	48.11 ± 0.59	96.25 ± 1.45	306.8 ± 0.95
5	82.58 ± 0.96	270.81 ± 0.73	84.53 ± 0.89	338.81 ± 0.43	14.90 ± 0.25	-
6a	83.69 ± 0.98	231.34 ± 0.45	60.65 ± 1.11	447.42 ± 0.58	47.81 ± 0.38	>500
6b	35.56 ± 0.53	>500	31.47 ± 0.36	>500	89.48 ± 0.11	335.6 ± 0.21
6c	98.10 ± 0.97	109.11 ± 0.79	96.43 ± 0.87	172.23 ± 0.57	86.75 ± 0.85	253.7 ± 0.95
6d	96.15 ± 1.12	161.83 ± 0.81	83.76 ± 0.87	302.31 ± 0.78	33.54 ± 0.65	-
6e	93.69 ± 0.84	87.53 ± 0.49	93.43 ± 0.96	133.93 ± 0.77	75.61 ± 0.96	351.9 ± 0.12
6f	81.94 ± 1.25	183.11 ± 0.89	86.10 ± 0.98	62.10 ± 0.52	79.80 ± 0.38	323.8 ± 0.45
6g	77.78 ± 0.95	206.77 ± 0.79	51.41 ± 0.66	494.23 ± 0.41	47.80 ± 0.63	>500
6h	85.48 ± 1.19	145.81 ± 0.93	86.71 ± 1.15	217.41 ± 0.92	70.15 ± 0.55	385.1 ± 0.54
6i	95.63 ± 1.14	168.11 ± 0.91	89.12 ± 0.81	135.92 ± 0.72	99.64 ± 1.85	73.12 ± 1.99
Control	91.29 ± 1.17[a]	0.04±0.0001[a]	82.82 ± 1.09[a]	0.85 ± 0.0001[a]	93.79 ± 1.27[b]	22.4 ± 1.3[b]

Note: IC_{50} values (concentration at which there is 50% enzyme inhibition) of compounds were calculated using EZ–Fit Enzyme kinetics software (Perella Scientific Inc. Amherst, USA). AChE = Acetyl cholinesterase BChE = Butyrylcholinesterase LOX = Lipoxygenase a = Eserine b = Baicalein

TABLE-2
PERCENTAGE INHIBITION AND MIC VALUES OF ANTIBACTERIAL ACTIVITY OF SYNTHESIZED MOLECULES

Com	*S. typhi (-)*		*E. coli (-)*		*P. aeroginosa (-)*		*B. subtili (+)*		*S. aureus (+)*	
	% age inhibition	MIC	% age inhibition	MIC	% age inhibition	MIC	% age inhibition	MIC	% age inhibition	MIC
3a	68.07±1.13	11.03±2.00	71.16±0.18	11.66±3.22	56.63±2.50	16.96±5.00	47.67±3.40	-	47.64±2.21	-
3b	53.27±2.47	16.08±3.64	53.96±1.52	18.32±2.76	49.63±4.50	-	41.07±0.67	-	46.57±0.57	-
3c	69.67±1.53	10.64±3.55	64.82±1.40	13.56±3.89	48.31±4.31	-	44.60±1.67	-	52.29±4.14	18.44±4.64
3d	61.33±1.20	11.55±1.82	55.98±3.05	14.49±4.05	51.50±1.25	19.33±4.17	46.80±1.73	-	59.43±4.71	13.83±1.98
3e	63.47±1.33	11.82±2.76	53.05±2.93	16.62±4.17	60.00±0.38	17.30±5.00	35.07±0.40	-	47.36±0.36	-
3f	50.20±1.27	19.70±4.18	44.82±3.60	-	43.94±1.94	-	32.33±2.60	-	38.07±0.93	-
3g	49.33±2.93	-	29.57±4.23	-	44.06±0.06	-	24.73±1.80	-	40.93±2.93	-
3h	67.47±0.00	10.85±4.73	61.40±2.26	14.83±2.33	58.94±0.19	16.99±5.00	38.53±0.40	-	55.43±4.71	16.01±2.73
3i	67.47±3.60	11.02±1.54	60.67±1.52	15.91±1.67	44.88±2.75	-	49.00±1.40	-	49.43±0.14	-
5	85.47±0.00	10.79±1.73	85.67±0.18	11.32±3.89	77.13±0.00	10.64±2.43	84.00±0.93	10.64±1.40	86.14±0.86	10.46±1.77
6a	54.40±3.47	11.98±2.09	44.76±4.98	-	48.00±1.00	-	30.73±0.33	-	37.50±3.93	-
6b	63.87±0.40	11.26±3.13	75.30±0.06	15.43±3.56	57.19±1.06	17.83±3.83	30.93±0.00	-	55.14±2.71	18.14±1.44
6c	67.73±0.13	10.24±2.52	69.33±1.40	10.69±3.72	54.06±1.44	16.58±3.33	61.87±4.27	12.87±1.28	59.14±0.57	14.80±2.31
6d	59.87±0.93	13.36±1.87	53.84±0.06	17.43±2.44	37.63±0.25	-	35.60±4.13	-	42.57±4.57	-
6e	58.40±1.33	14.76±3.73	43.96±3.43	-	57.31±1.81	17.41±5.00	41.73±4.27	-	56.93±2.36	17.52±2.00
6f	78.33±1.13	10.98±1.76	70.43±4.72	12.19±2.39	65.13±1.38	13.05±4.50	60.00±1.20	12.52±2.36	62.86±4.00	12.11±3.91
6g	66.93±0.40	11.94±3.00	64.15±1.34	10.82±1.06	58.13±0.63	18.47±4.08	42.53±1.87	-	51.00±1.57	17.44±3.27
6h	81.80±0.87	10.43±2.64	82.01±1.16	10.83±1.41	67.13±0.25	10.46±2.67	69.20±2.27	11.65±1.42	60.93±1.36	14.22±2.42
6i	80.80±0.40	10.88±4.09	73.54±0.85	11.90±4.22	65.88±1.00	12.39±1.17	73.27±1.40	11.41±1.47	69.93±2.79	11.25±2.12
Ci	91.65±1.04	9.22±1.36	90.98±1.43	8.79±2.00	90.46±1.99	8.93±2.42	89.90±0.79	9.43±1.87	92.04±1.44	9.04±1.50

Note: Minimum inhibitory concentration (MIC) was measured with suitable dilutions (5-30 μg/ well) and results were calculated using EZ-FitPerrella Scientific Inc. Amherst USA software; Ci = Ciprofloxacin.

were confirmed through spectral analysis as depicted in experimental section. The physical data for the final product **6a** is given in experimental section. The HR-MS provided a $[M]^+$ peak at 454.4975 (Calcd. for $C_{23}H_{22}N_2O_6S$; 454.5031). The molecular formula $C_{23}H_{22}N_2O_6S$ was also supported by molecular ion peak at *m/z* 454 in EIMS and the number of protons in its ^1H NMR spectrum. The peaks of EIMS spectrum which well supported the molecule, were *m/z* 150, 141 and 121 for the cations of 2,3-dihydro-1,4-benzodioxin-6-amino group, phenylsulfonyl group and 4-(methylamino)phenoxy group respectively. The IR spectrum supported the structure by two characteristics absorption bands at 3462 cm^{-1} and 1381

cm^{-1} for N-H (stretching) of amide and S=O (stretching) of sulfonyl group, respectively. Benzene sulfonyl ring was confirmed by two signals in the aromatic region of ^{1}H NMR spectrum appearing at δ 7.64 (dd, *J* = 7.8, 1.8 Hz, 2H, H-2' & H-6') and 7.51-7.41 (m, 3H, H-3' to H-5'). The benzodioxine ring was supported by four signals, three in aromatic region and one in aliphatic region, at δ 7.17 (d, *J* = 2.4, 1H, H-5'''), 7.02 (dd, *J* = 8.4, 1.2 Hz, 1H, H-7'''), 6.99 (d, *J* = 7.5 Hz, 1H, H-8''') and 4.21 (s, 4H, CH_2-2''' & CH_2-3'''). The two doublets resonating at δ 6.97 (d, *J* = 8.1 Hz, 2H, H-2 & H-6) and 6.78 (d, *J* = 8.7 Hz, 2H, H-3 & H-5) were assigned to the protons of 1,4-disubstituted phenyl ring. In the aliphatic region of the ^{1}H NMR spectrum, the two singlets, appearing at δ 3.92 (s, 2H, CH_2-2'''') and 3.14 (s, 3H, CH_3-1'') in 2:3 ratio, indicated the presence of two methylene and three methyl protons in the molecule. The proposed structure of **6a** was corroborated and named, 2-{4-[methyl(phenylsulfonyl) amino]phenoxy}-*N*-(2,3-dihydro-1,4-benzodioxin-6-yl)-acetamide. Likewise, the structures of other synthesized compounds were characterized. The mass fragmetation pattern of **6a** is given in Fig. 1.

Enzyme inhibition activity: The results of screening against all the three enzymes are presented in Table-1 in the

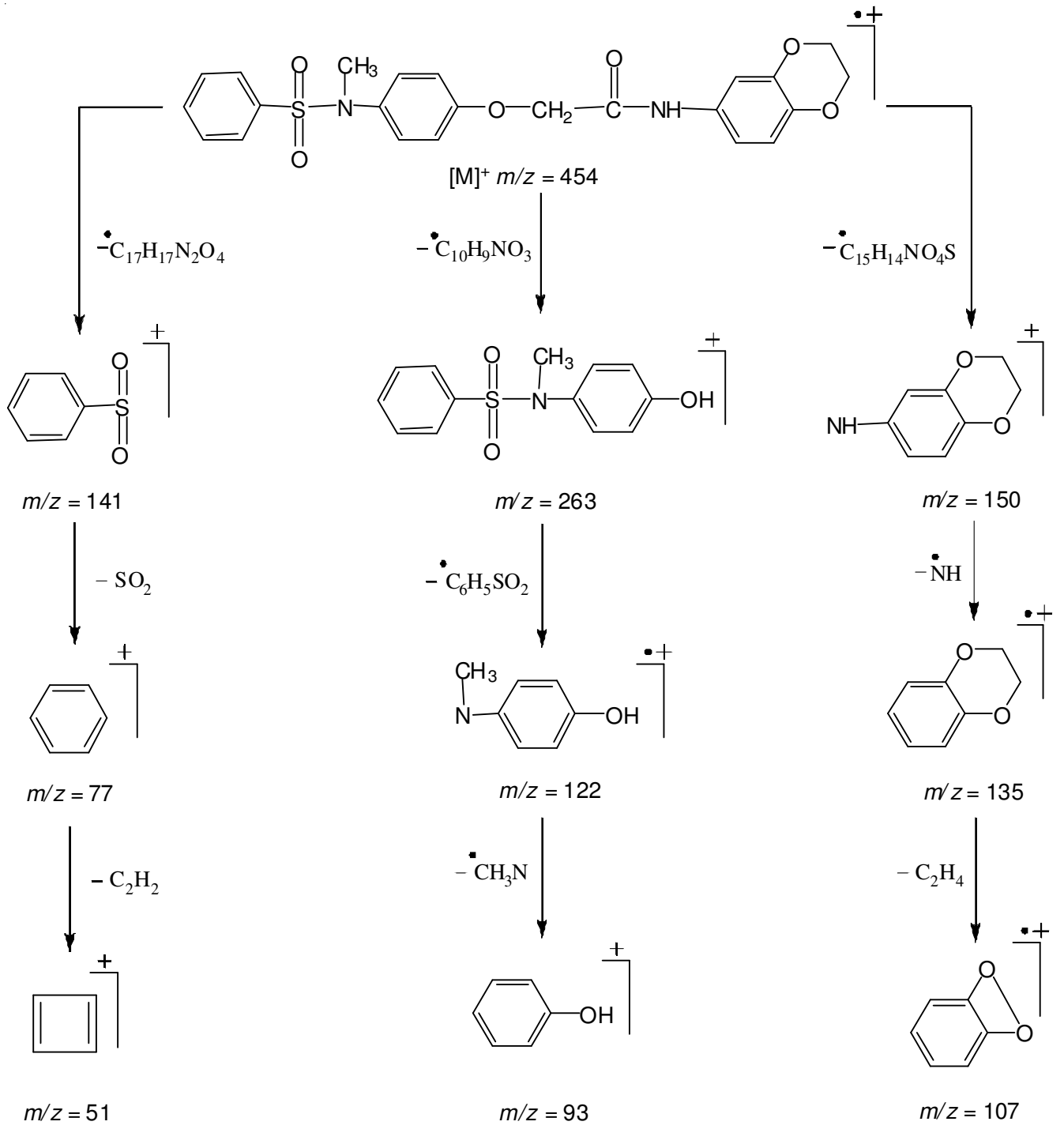


Fig. 1. Mass fragmentation pattern of 2-{4-[methyl(phenylsulfonyl)amino]phenoxy}-*N*-(2,3-dihydro-1,4-benzodioxin-6-yl)acetamide (**6a**)

form of % age inhibition and IC_{50} values. The screening of synthesized molecules against acetyl cholinesterase enzyme showed that all the molecules exhibited promising inhibitory action and **3b** was the most prominent with the lowest IC_{50} value of 21.62 ± 0.53 µM relative to the reference standard, eserine, with IC_{50} value of 0.04 ± 0.0001 µM. Only **6b** molecule remained inactive as evident from IC_{50} value. Butyryl cholinesterase enzyme was also promisingly inhibited by all the molecules except **6b**. **3i** was the most active against this enzyme by the IC_{50} value of 48.11 ± 0.59 µM relative to reference standard, eserine, with IC_{50} value of 0.85 ± 0.0001 µM. The most potent molecules against both the cholinesterase enzymes were **3c** and **3i** with the structural features of *p*-substituted tertiary methyl and naphthyl group interacting at large area respectively. Against lipoxygenase enzyme, some were inactive and most of them showed moderate inhibitory action. The most potent molecule against this enzyme was **6i** with IC_{50} value of 73.12 ± 1.99 µM relative to baicalein with IC_{50} value of 22.4 ± 1.3 µM.

Antibacterial activity: All the synthesized molecules were screened against three Gram-negative and two Gram-positive bacterial strains and were found to be moderate inhibitors. The results are tabulated as (%) inhibition and MIC values in Table-2. Most of the compounds showed activities against the both Gram-positive bacterial strains except a few. Almost all the compounds showed the same inhibition potential against *S. typhi* and *E. coli*, as that of the reference standard, ciprofloxacin used. *P. aeroginosa* and *S. aureus* were inhibited by the majority of the molecules with 50 % inhibitory action as that of the reference used. The synthesized molecules, **5**, **6f**, **6h** and **6i** were active against all the bacterial strains with much low MIC values, credibly because of the presence of bromo group at 2nd carbon of acetyl group, bromo group at 4th position of phenyl group, two chloro groups at 2nd and 5th position of phenyl group and naphthyl group, respectively. The aromatic naphthyl rings are responsible for more interaction and the halogens in better positions are creditworthy for the antibacterial inhibition potential.

Computational analysis of potent compounds against acetyl cholinesterase (AchE) and butyryl cholinesterase (BChE): The synthesized compounds were computationally docked against the active site of acetyl cholinesterase and butyryl cholinesterase to find out their binding modes. All the compounds showed good binding pattern, **3b** is discussed for acetyl cholinesterase and **3c** for butyryl cholinesterase as below.

Interaction analysis of compound 3b against acetyl cholinesterase: One of the potent compound **3b** showed very decent interaction with the active site of the target protein acetyl cholinesterase. A total of six interactions were observed with five residues of the active sites. Tyr 70, Asp72, Trp 84 and Asn 85 made interaction with the phenol ring. However, Trp 84 also interacts with the toluene moiety through arene-hydrogen bonding. Ser 122 interacts with sulfonamide group and makes the interaction of compound stronger (Fig. 2). So, in this interaction analysis we observed that three different moieties of compound are contributing in the interaction between ligand and protein.

Interaction analysis of compound 3c against butyryl cholinesterase: The interaction analysis of compound **3c** in

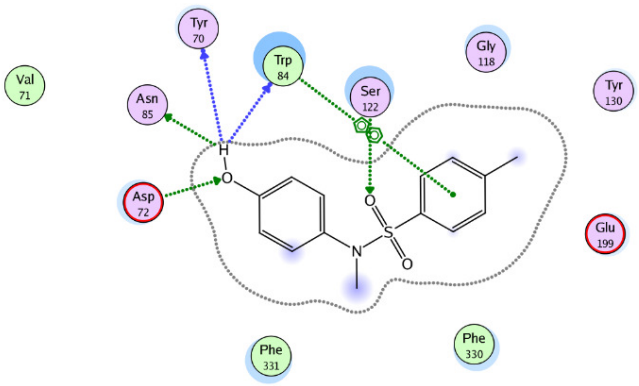


Fig. 2. 2D interaction analysis of docked compound **3b** against acetyl cholinesterase

case of butyryl cholinesterase revealed that two important moieties are contributing in the interactions. Asn 85 interacts with the phenol moiety whereas; His126 interacts with sulfonamide group directly as well as through water molecule (Fig. 3).

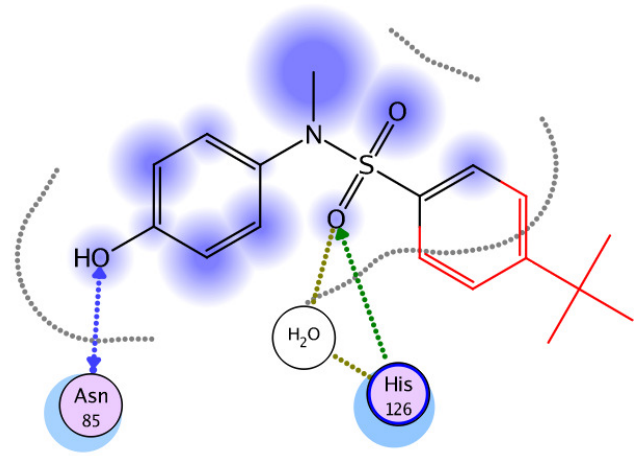


Fig. 3. 2D interaction analysis of compound **3c** with butyryl cholinesterase

Computational analysis of a potent compound 6i against lipoxygenase (LOX): All the compounds were computationally docked against lipoxygenase as well, a similar sort of interactions was observed in case of lipoxygenase. Here, compound **6i** is shown in Fig. 4. A total of six interactions were observed with five residues of the binding site. Val 566 interacts with oxygen of sulfonamide moiety, whereas rest of other five interactions was observed with acetamide moiety. His513, His518, Gln514 and Gln716 are other interacting residues of the active site shown in Fig. 4.

Conclusion

All the compounds were synthesized in good yields by the depicted methods in experimental section. The proposed structures were corroborated by the spectroscopic analysis using IR, ^{1}H NMR and EIMS techniques. All the synthesized compounds were evaluated for the enzyme inhibition and antibacterial activities. The IC_{50} values of these molecules rendered them better inhibitors of cholinesterase enzymes and moderately less inhibitors of lipoxygenase enzyme. The MIC values of taken into account molecules against the different

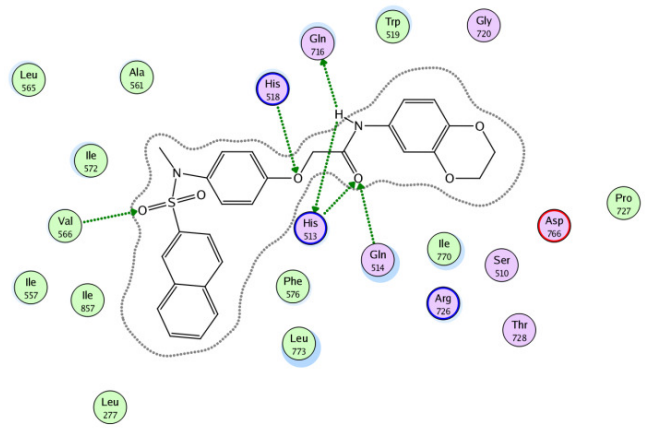


Fig. 4. 2D interaction analysis of compound **6i** with lipoxygenase

bacterial strains, showed them potent against two of three Gram-negative bacterial strains and moderately good against two Gram-positive bacterial strains. All these biological results depicted the synthesized molecules as an important pathway for the pharmacological industries in drug designing.

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